

## ab176757 - CytoPainter Phalloidin-iFluor 594 Reagent

For staining actin filaments (F-actin) in formaldehyde-fixed cells and tissues

This product is for research use only and is not intended for diagnostic use

### Storage and Stability:

Store reagent at -20°C in the dark immediately upon receipt. Reagent has a storage time of 6 months from receipt.

### Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phalloidin-iFluor 594 Conjugate	300 tests	-20°C	-20°C

### Materials Required, Not Supplied

These materials are not included, but will be required to successfully perform this assay:

- Fluorescence microscope fitted with a filter capable of detecting fluorescence at Ex/Em = 590/618 nm
- PBS
- PBS + 1% BSA
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96 well plate with clear flat bottom, preferably black (if performing assay in microplate format). Use a poly-D-lysine coated plate for suspension cells
- 3-4% formaldehyde solution in PBS – for fixation step
- (Optional) Triton X-100: to add to PBS to increase permeability
- (Optional) DNA labeling reagent with different excitation/emission spectra to phalloidin-iFluor conjugate
- (Optional) Mounting media – we recommend Fluoroshield Mounting Media (ab104135)

**Reagent Preparation** Briefly centrifuge small vial at low speed prior to opening.

### **Phalloidin-iFluor 594 conjugate (1000X stock):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot stock solution so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Avoid repeated freeze-thaw cycles.

**Δ Note:** Phalloidin is toxic. Although the amount of toxin present in the vial could be lethal only to a mosquito (LD<sub>50</sub> of phalloidin = 2 mg/kg), it should be handled with care.

### Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.

- We recommend that you assay all controls and samples in duplicate.
- The protocol described in this section has been optimized for staining in 96-well plate. Staining can also be performed in cover-slips inside a petri dish. In that case, you will need to modify the volume of the staining solution accordingly to the final volume.
- This protocol can be combined with an antibody-based staining. Phalloidin conjugate can be added either during the primary antibody incubation or during the secondary antibody / DNA staining incubation step.

**Δ Note:** The optimal concentration and incubation time of the Phalloidin-iFluor 594 conjugate will vary depending on the specific application. The staining conditions may be modified according to the particular cell type and/or the permeability of the cells or tissues to the probe.

### **1. Prepare 1X Phalloidin-iFluor 594 Working solution:**

- 1.1.1 Add 1 µL of the 1000X Phalloidin conjugate Stock solution in 1 mL of PBS + 1% BSA and mix well by pipetting up and down. This makes enough staining solution for 10 wells (100 µL/well).

**Δ Note:** PBS without BSA can also be used to prepare working solution. Addition of BSA is preferred as it will minimize the chances of phalloidin sticking to the tube.

**Δ Note:** Do not store diluted 1X working solution. Simply make enough volume for the number of samples required.

- 1.1.2 Proceed to step 10.2 for adherent cell staining protocol or step 10.3 for suspension cell staining protocol.

### **2. Adherent cell staining:**

- 1.2.1 Grow cells in a 96 well black wall/clear bottom plate with the appropriate culture medium till they reach desired confluence (recommendation: 70-80%).

**Δ Note:** cells can also be grown cover-slips inside a petri dish. In that case, you will need to modify the volume of the staining solution accordingly to the final volume.

- 1.2.2 Aspirate cell culture medium carefully to avoid dislodging any cells from the plate.
- 1.2.3 Wash once in PBS.
- 1.2.4 Formaldehyde fixation: incubate cells in 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.

**Δ Note:** avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

- 1.2.5 Aspirate staining solution carefully and wash fixed cells 2-3 times in PBS.
- 1.2.6 Optional: add 0.1% Triton X-100 in PBS into the fixed cells for 3-5 minutes to increase permeability. Wash permeabilized cells 2-3 times in PBS.
- 1.2.7 Add 100 µL of 1X Phalloidin conjugate working solution (Step 10.1) to each well of fixed cells. Incubate cells at room temperature for 20-90 minutes.

**Δ Note:** if using, you can add DNA staining dye at this point.

- 1.2.8 Rinse cells gently 2-3 times with PBS to remove excess phalloidin conjugate.
- 1.2.9 Add mounting media (to preserve fluorescence) and seal (if using coverslips).
- 1.2.10 Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 590/618 nm.

### **3. Suspension cell staining:**

- 1.3.1 Grow cells in the appropriate culture vessel until they reach the desired confluence (70-80%).

**Δ Note:** Suspension cells may be attached to microplate or coverslips that have been treated with poly-D-lysine can be stained following the procedure for adherent cells (Step 10.2).

- 1.3.2 Centrifuge suspension cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
- 1.3.3 Resuspend the cell pellets gently in pre-warmed (37°C) growth medium and transfer to microplate or coverslips.
- 1.3.4 Aspirate cell culture medium carefully to avoid dislodging any cells from the plate. Wash once in PBS.
- 1.3.5 Formaldehyde fixation: incubate cells in 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.

**Δ Note:** avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

- 1.3.6 Aspirate staining solution carefully and wash fixed cells 2- 3 times in PBS.
- 1.3.7 Optional: add 0.1% Triton X-100 in PBS into the fixed cells for 3- 5 minutes to increase permeability. Wash permeabilized cells 2-3 times in PBS.
- 1.3.8 Add 100 µL of 1X Phalloidin conjugate working solution (Step 10.1) to each well of fixed cells. Incubate cells at room temperature for 20-90 minutes.

**Δ Note:** if using, you can add DNA staining dye at this point.

- 1.3.9 Rinse cells gently 2-3 times with PBS to remove excess phalloidin conjugate.
- 1.3.10 Add mounting media (to preserve fluorescence) and seal (if using coverslips).
- 1.3.11 Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 590/618 nm.

### **Data Analysis**

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

### **Troubleshooting**

Problem	Reason	Solution
Actin filaments not sufficiently stained	Low dye concentration / Incubation time insufficient	Increase dye concentration and/or incubation time
	Cells analysed at incorrect wavelength	Ensure you are using the appropriate filter settings
Cells do not appear healthy	Cells require serum to remain healthy	Add serum (2-10% range) to stain and wash solutions
Nuclear counterstain is too bright	Different microscopes, cameras and filters may make some signals appear very bright	Reduce concentration of nuclear counterstain or shorten exposure time

### **Technical Support**

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